



10/797,262
SEQUENCE LISTING

<110> East Tennessee State University Research Foundation
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Vellore, Jashree

<120> RNA-DEPENDENT DNA POLYMERASE FROM GEOBACILLUS STEAROTHERMOPHILUS

<130> 2826067.000002

<140> 10/797,262

<141> 2004-03-10

<160> 12

<170> PatentIn version 3.3

<210> 1

<211> 1263

<212> DNA

<213> Geobacillus stearothermophilus

<220>

<221> CDS

<222> (1)..(1263)

<400> 1

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Met Ala Leu Leu Glu Arg Ile Leu Ala Arg Asp Asn Leu Ile Thr Ala	
1 5 10 15	
ctc aaa cgg gtc gaa gcc aac caa gga gca ccg gga atc gac gga gta	96
Leu Lys Arg Val Glu Ala Asn Gln Gly Ala Pro Gly Ile Asp Gly Val	
20 25 30	
tca acc gat caa ctc cgt gat tac atc cgc gct cac tgg agc acg atc	144
Ser Thr Asp Gln Leu Arg Asp Tyr Ile Arg Ala His Trp Ser Thr Ile	
35 40 45	
cgc gcc caa ctc ttg gcg gga acc tac cgg ccg gcg cct gtc cgc agg	192
Arg Ala Gln Leu Leu Ala Gly Thr Tyr Arg Pro Ala Pro Val Arg Arg	
50 55 60	
gtc gga atc ccg aaa ccg ggc ggc ggc aca cgg cag cta ggc att ccc	240
Val Gly Ile Pro Lys Pro Gly Gly Gly Thr Arg Gln Leu Gly Ile Pro	
65 70 75 80	
acc gtg gtg gac cgg ctg atc caa caa gcc att ctt caa gaa ctc aca	288
Thr Val Val Asp Arg Leu Ile Gln Gln Ala Ile Leu Gln Glu Leu Thr	
85 90 95	
ccc att ttc gat cca gac ttc tcc cct tcc agc ttc gga ttc cgt ccg	336
Pro Ile Phe Asp Pro Asp Phe Ser Pro Ser Ser Phe Gly Phe Arg Pro	
100 105 110	
ggc cgt aac gcc cac gat gcc gtg cgg caa gcg caa ggc tac atc cag	384
Gly Arg Asn Ala His Asp Ala Val Arg Gln Ala Gln Gly Tyr Ile Gln	
115 120 125	

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gaa Glu	ggg Gly	tat Tyr	cgg Arg	tac Tyr	gtg Val	gtc Val	gac Asp	atg Met	gac Asp	ctg Leu	gaa Glu	aag Lys	ttc Phe	ttt Phe	gat Asp	432
	130					135					140					
cgg Arg	gtc Val	aac Asn	cat His	gac Asp	atc Ile	ttg Leu	atg Met	agt Ser	cgg Arg	gtg Val	gcc Ala	cga Arg	aaa Lys	gtc Val	aag Lys	480
145					150					155					160	
gat Asp	aaa Lys	cgc Arg	gtg Val	ctg Leu	aaa Lys	ctg Leu	atc Ile	cgt Arg	gcc Ala	tac Tyr	ctg Leu	caa Gln	gcc Ala	ggc Gly	gtt Val	528
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atg Met	atc Ile	gaa Glu	ggg Gly	gtg Val	aag Lys	gtg Val	cag Gln	acg Thr	gag Glu	gaa Glu	ggg Gly	acg Thr	ccg Pro	caa Gln	ggc Gly	576
			180					185					190			
ggc Gly	ccc Pro	ctc Leu	agc Ser	ccc Pro	ctg Leu	ctg Leu	gcg Ala	aac Asn	atc Ile	ctt Leu	ctc Leu	gac Asp	gat Asp	tta Leu	gac Asp	624
		195					200					205				
aag Lys	gaa Glu	ttg Leu	gag Glu	aag Lys	cga Arg	gga Gly	ttg Leu	aaa Lys	ttc Phe	tgc Cys	cgt Arg	tac Tyr	gca Ala	gat Asp	gac Asp	672
	210					215					220					
tgc Cys	aac Asn	atc Ile	tat Tyr	gtg Val	aaa Lys	agt Ser	ctg Leu	cgg Arg	gca Ala	gga Gly	caa Gln	cgg Arg	gtg Val	aaa Lys	caa Gln	720
225					230					235					240	
agc Ser	atc Ile	caa Gln	cgg Arg	ttc Phe	ttg Leu	gag Glu	aaa Lys	acg Thr	ctc Leu	aaa Lys	ctc Leu	aaa Lys	gta Val	aac Asn	gag Glu	768
				245					250					255		
gag Glu	aaa Lys	agt Ser	gcg Ala	gtg Val	gac Asp	cgc Arg	ccg Pro	tgg Trp	aaa Lys	cgg Arg	gcc Ala	ttt Phe	ctg Leu	ggg Gly	ttt Phe	816
			260					265					270			
agc Ser	ttc Phe	aca Thr	ccg Pro	gaa Glu	cga Arg	aaa Lys	gcg Ala	cga Arg	atc Ile	cgg Arg	ctc Leu	gcc Ala	cca Pro	agg Arg	tcg Ser	864
		275					280					285				
att Ile	caa Gln	cgt Arg	ctg Leu	aaa Lys	cag Gln	cgg Arg	att Ile	cga Arg	cag Gln	ctg Leu	acc Thr	aac Asn	cca Pro	aac Asn	tgg Trp	912
	290					295					300					
agc Ser	ata Ile	tcg Ser	atg Met	cca Pro	gaa Glu	cga Arg	att Ile	cat His	cgc Arg	gtc Val	aat Asn	caa Gln	tac Tyr	gtc Val	atg Met	960
305					310					315					320	
gga Gly	tgg Trp	atc Ile	ggg Gly	tat Tyr	ttt Phe	cgg Arg	ctc Leu	gtc Val	gaa Glu	acc Thr	ccg Pro	tct Ser	gtc Val	ctt Leu	cag Gln	1008
				325					330					335		
acc Thr	atc Ile	gaa Glu	gga Gly	tgg Trp	att Ile	cgg Arg	agg Arg	agg Arg	ctt Leu	cga Arg	ctc Leu	tgt Cys	caa Gln	tgg Trp	ctt Leu	1056
			340				345						350			
caa Gln	tgg Trp	aaa Lys	cgg Arg	gtc Val	aga Arg	acc Thr	aga Arg	atc Ile	cgt Arg	gag Glu	tta Leu	aga Arg	gcg Ala	ctg Leu	ggg Gly	1104

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355	360	365	
ctg aaa gag aca gcg gtg atg gag atc gcc aat acc cga aaa gga gct			1152
Leu Lys Glu Thr Ala Val Met Glu Ile Ala Asn Thr Arg Lys Gly Ala			
370	375	380	
tg g cga aca acg aaa acg ccg caa ctc cac cag gcc ctg ggc aaa acc			1200
Trp Arg Thr Thr Lys Thr Pro Gln Leu His Gln Ala Leu Gly Lys Thr			
385	390	395	400
tac tgg acc gct caa ggg ctc aag agt ttg acg caa cga tat ttc gaa			1248
Tyr Trp Thr Ala Gln Gly Leu Lys Ser Leu Thr Gln Arg Tyr Phe Glu			
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Leu Arg Gln Gly			
420			

<210> 2
 <211> 420
 <212> PRT
 <213> Geobacillus stearothermophilus

<400> 2

Met Ala Leu Leu Glu Arg Ile Leu Ala Arg Asp Asn Leu Ile Thr Ala
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Leu Lys Arg Val Glu Ala Asn Gln Gly Ala Pro Gly Ile Asp Gly Val
 20 25 30

Ser Thr Asp Gln Leu Arg Asp Tyr Ile Arg Ala His Trp Ser Thr Ile
 35 40 45

Arg Ala Gln Leu Leu Ala Gly Thr Tyr Arg Pro Ala Pro Val Arg Arg
 50 55 60

Val Gly Ile Pro Lys Pro Gly Gly Gly Thr Arg Gln Leu Gly Ile Pro
 65 70 75 80

Thr Val Val Asp Arg Leu Ile Gln Gln Ala Ile Leu Gln Glu Leu Thr
 85 90 95

Pro Ile Phe Asp Pro Asp Phe Ser Pro Ser Ser Phe Gly Phe Arg Pro
 100 105 110

Gly Arg Asn Ala His Asp Ala Val Arg Gln Ala Gln Gly Tyr Ile Gln
 115 120 125

Glu Gly Tyr Arg Tyr Val Val Asp Met Asp Leu Glu Lys Phe Phe Asp

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130		135		140											
Arg 145	Val	Asn	His	Asp	Ile 150	Leu	Met	Ser	Arg	Val 155	Ala	Arg	Lys	Val	Lys 160
Asp	Lys	Arg	Val	Leu 165	Lys	Leu	Ile	Arg	Ala 170	Tyr	Leu	Gln	Ala	Gly 175	Val
Met	Ile	Glu	Gly 180	Val	Lys	Val	Gln	Thr 185	Glu	Glu	Gly	Thr	Pro 190	Gln	Gly
Gly	Pro	Leu 195	Ser	Pro	Leu	Leu	Ala 200	Asn	Ile	Leu	Leu	Asp 205	Asp	Leu	Asp
Lys	Glu 210	Leu	Glu	Lys	Arg	Gly 215	Leu	Lys	Phe	Cys	Arg 220	Tyr	Ala	Asp	Asp
Cys 225	Asn	Ile	Tyr	Val	Lys 230	Ser	Leu	Arg	Ala	Gly 235	Gln	Arg	Val	Lys	Gln 240
Ser	Ile	Gln	Arg	Phe 245	Leu	Glu	Lys	Thr	Leu 250	Lys	Leu	Lys	Val	Asn 255	Glu
Glu	Lys	Ser	Ala 260	Val	Asp	Arg	Pro	Trp 265	Lys	Arg	Ala	Phe	Leu 270	Gly	Phe
Ser	Phe	Thr 275	Pro	Glu	Arg	Lys	Ala 280	Arg	Ile	Arg	Leu	Ala 285	Pro	Arg	Ser
Ile	Gln 290	Arg	Leu	Lys	Gln	Arg 295	Ile	Arg	Gln	Leu	Thr 300	Asn	Pro	Asn	Trp
Ser 305	Ile	Ser	Met	Pro	Glu 310	Arg	Ile	His	Arg	Val 315	Asn	Gln	Tyr	Val	Met 320
Gly	Trp	Ile	Gly	Tyr 325	Phe	Arg	Leu	Val	Glu 330	Thr	Pro	Ser	Val	Leu 335	Gln
Thr	Ile	Glu	Gly 340	Trp	Ile	Arg	Arg	Arg 345	Leu	Arg	Leu	Cys	Gln 350	Trp	Leu
Gln	Trp	Lys 355	Arg	Val	Arg	Thr	Arg 360	Ile	Arg	Glu	Leu	Arg 365	Ala	Leu	Gly

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Leu Lys Glu Thr Ala Val Met Glu Ile Ala Asn Thr Arg Lys Gly Ala
 370 375 380

Trp Arg Thr Thr Lys Thr Pro Gln Leu His Gln Ala Leu Gly Lys Thr
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Tyr Trp Thr Ala Gln Gly Leu Lys Ser Leu Thr Gln Arg Tyr Phe Glu
 405 410 415

Leu Arg Gln Gly
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<210> 3
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 <212> DNA
 <213> Artificial

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 <223> Plasmid construct

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 <221> misc_feature
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 aacgcattctt agcgagagac aacctcatca cggcgctcaa acgggtcgaa gccaaccaag 180
 gagcaccggg aatcgacgga gtatcaaccg atcaactccg tgattacatc cgcgctcact 240
 ggagcacgat ccgcgccccaa ctcttgccgg gaacctaccg gccggcgccg gtccgcaggg 300
 tcggaatccc gaaaccgggc ggcggcacac ggcagctagg cattcccacc gtggtggacc 360
 ggctgatcca acaagccatt cttcaagaac tcacacccat ttctgatcca gacttctccc 420
 cttccagctt cggattccgt ccggggccgta acgcccacga tgccgtgcgg caagcgcaag 480
 gctacatcca ggaagggtat cggtacgtgg tcgacatgga cctggaaaag ttctttgatc 540
 gggtaacca tgacatcttg atgagtcggg tggcccgaag agtcaaggat aaacgcgtgc 600
 tgaaactgat ccgtgcctac ctgcaagccg gcgttatgat cgaaggggtg aaggtgcaga 660
 cggaggaagg gacgccgcaa ggcggccccc tcagccccct gctggcgaac atccttctcg 720
 acgatttaga caaggaattg gagaagcgag gattgaaatt ctgccgttac gcagatgact 780
 gcaacatcta tgtgaaaagt ctgcgggcag gacaacgggt gaaacaaagc atccaacggt 840

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tcttggagaa aacgctcaaa ctcaaagtaa acgaggagaa aagtgcggtg gaccgcccgt	900
ggaaacgggc ctttctgggg tttagcttca caccggaacg aaaagcgcga atccggctcg	960
ccccaaggtc gattcaacgt ctgaaacagc ggattcgaca gctgaccaac ccaaactgga	1020
gcatatcgat gccagaacga attcatcgcg tcaatcaata cgtcatggga tggatcgggt	1080
atcttcggct cgtcgaaacc ccgtctgtcc ttcagaccat cgaaggatgg attcggagga	1140
ggcttcgact ctgtcaatgg cttcaatgga aacgggtcag aaccagaatc cgtgagttaa	1200
gagcgctggg gctgaaagag acagcggtga tggagatcgc caatacccga aaaggagctt	1260
ggcgaacaac gaaaacgccg caactccacc aggccctggg caaacctac tggaccgctc	1320
aagggctcaa gagtttgacg caacgatatt tcgaactccg tcaaggttga	1370

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 <212> DNA
 <213> Artificial

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 <223> Nucleotide primer containing NdeI restriction site

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 <221> primer_bind
 <222> (1)..(32)

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<210> 5
 <211> 28
 <212> DNA
 <213> Artificial

<220>
 <223> Nucleotide primer containing BamHI restriction site

<400> 5	
aatggatccg ctggcgaaca tccttctc	28

<210> 6
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 <212> DNA
 <213> Artificial

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 <223> Nucleotide primer containing PstI restriction site

<220>
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<222> (1)..(29)

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attactgcag agcgggtccag taggttttg

29

<210> 7
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<212> DNA
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<223> Nucleotide primer containing HindIII restriction site

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<222> (1)..(31)

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<210> 8
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<212> PRT
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<223> Amino acid sequence of fusion protein

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<222> (1)..(455)

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Arg Gly Ser His Met Arg Gln Asp Leu Asn Leu Ile Pro Arg Lys Glu
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Lys Ile Thr Met Ala Leu Leu Glu Arg Ile Leu Ala Arg Asp Asn Leu
35 40 45

Ile Thr Ala Leu Lys Arg Val Glu Ala Asn Gln Gly Ala Pro Gly Ile
50 55 60

Asp Gly Val Ser Thr Asp Gln Leu Arg Asp Tyr Ile Arg Ala His Trp

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Leu Gly Phe Ser Phe Thr Pro Glu Arg Lys Ala Arg Ile Arg Leu Ala
 305 310 315 320

Pro Arg Ser Ile Gln Arg Leu Lys Gln Arg Ile Arg Gln Leu Thr Asn
 325 330 335

Pro Asn Trp Ser Ile Ser Met Pro Glu Arg Ile His Arg Val Asn Gln
 340 345 350

Tyr Val Met Gly Trp Ile Gly Tyr Phe Arg Leu Val Glu Thr Pro Ser
 355 360 365

Val Leu Gln Thr Ile Glu Gly Trp Ile Arg Arg Arg Leu Arg Leu Cys
 370 375 380

Gln Trp Leu Gln Trp Lys Arg Val Arg Thr Arg Ile Arg Glu Leu Arg
 385 390 395 400

Ala Leu Gly Leu Lys Glu Thr Ala Val Met Glu Ile Ala Asn Thr Arg
 405 410 415

Lys Gly Ala Trp Arg Thr Thr Lys Thr Pro Gln Leu His Gln Ala Leu
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Gly Lys Thr Tyr Trp Thr Ala Gln Gly Leu Lys Ser Leu Thr Gln Arg
 435 440 445

Tyr Phe Glu Leu Arg Gln Gly
 450 455

<210> 9
 <211> 25
 <212> DNA
 <213> Artificial

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 <223> Primer sequence

<220>
 <221> prim_transcript
 <222> (1)..(25)

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25

<210> 10
 <211> 25

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<213> Artificial

<220>
<223> Primer sequence

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<221> prim_transcript
<222> (1)..(25)

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25

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<211> 24
<212> DNA
<213> Artificial

<220>
<223> Primer sequence

<220>
<221> prim_transcript
<222> (1)..(24)

<400> 11
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24